Effects of angiotensin II type-1 receptor blocker losartan on age-related cardiovascular risk in spontaneously hypertensive rats

Nevena Mihailović-Stanojević, Zoran Miloradović, Jelica Grujić-Milanović, Milan Ivanov and Djurdjica Jovović

Institute for Medical Research, University of Belgrade, Belgrade, Serbia

Abstract. Ageing and hypertension are the major risk factors for the development of cardiovascular and renal diseases, and the renin-angiotensin system has been shown responsible for these pathologies. Thus, the aim of this study was to compare the effects of losartan, angiotensin II type-1 receptor blocker, on systolic (SBP), diastolic (DBP), and mean (MBP) blood pressure, pulse pressure (PP) and heart rate as well as regional haemodynamics, cardiac hypertrophy and biochemical parameters in adult (L_9: 9-month-old) and aged (L_{18}: 18-month-old) spontaneously hypertensive rats (SHRs). Aged match untreated SHRs served as controls (U_9: 9-month-old and U_{18}: 18-month-old). Aortal blood flow and resistance were significantly improved by losartan treatment in L_9 vs. U_9 ($p < 0.05$). In aged SHRs, losartan significantly reduced SBP, MBP, PP, right ventricle weight index, and improved age-related impairment of left ventricular weight index (U_{18}: 4.21 ± 0.09 mg/g vs. U_9: 3.54 ± 0.34 mg/g, $p < 0.05$ and vs. L_{18}: 3.65 ± 0.07 mg/g, $p < 0.001$), carotid, renal, and aortal vascular resistance, and glomerular filtration rate (U_{18}: 2.75 ± 0.27 ml/min/kg vs. U_9: 4.84 ± 0.85 ml/min/kg, $p < 0.05$ and vs. L_{18}: 3.65 ± 0.07 ml/min/kg, $p < 0.05$). These results demonstrate significantly impaired systemic and regional haemodynamics and left ventricular hypertrophy in old SHRs. Losartan decreased age and hypertension associated cardiovascular risk by decreasing vascular resistance and pressure overload, ventricular hypertrophy, and preserving kidney function.

Key words: Hypertension — Ageing — Cardiovascular risk — Losartan

Introduction

Cardiovascular complications, associated with ageing and/or hypertension, are caused by alterations in vascular structure and function (Taddei et al. 1997). High blood pressure (BP) is a powerful cardiovascular (CV) risk factor that acts on the arterial wall and is responsible in part for various CV events, such as cerebrovascular accidents and ischemic heart disease (Safar et al. 2003). Systolic and diastolic dysfunctions are independent CV risk factors in the elderly patients, while pulse pressure (PP) is a powerful predictor of CV events in this population (Van Bortel et al. 2001; Fyhrquist et al. 2005). In essential hypertension haemodynamic alterations characterized by increased

resistance in peripheral circulation are a consequence of structural, mechanical and functional changes in peripheral vasculature. Advanced age is associated with many CV pathophysiological states, including arteriosclerosis (Iwamoto et al. 2000), stroke (Arboix et al. 2006), heart failure and coronary artery disease (Susic et al. 1999b), and progressive renal damage (Mihailovic-Stanojevic et al. 2003). Age-associated remodeling of the walls of large arteries of rodents, nonhuman primates and humans includes luminal dilation, intimal and medial thickening, vascular stiffening, and endothelial dysfunction (Lakatta 2003). In addition, increase in intima-media thickness of the common carotid artery that occurred with ageing are associated with a number of CV complications and therefore can be predictive and independent factor of CV risk (Ariff et al. 2002). Left ventricular hypertrophy (LVH), ventricular fibrosis and impaired ventricular performances are cardiac manifestations of both hypertension and ageing, and components of the ventricle are affected in hyperten-

Correspondence to: Nevena Mihailović-Stanojević, Department of Cardiovascular Physiology, Institute for Medical Research, University of Belgrade, Dr. Subotića 4, P.O.Box 102, 11129 Belgrade, Serbia
E-mail: nevena@imi.bg.ac.rs

sive heart disease as well as in ageing heart (Varagic et al. 2001a). The spontaneously hypertensive rats (SHRs) have been extensively studied as a model of cardiac hypertrophy that results from genetically-induced pressure overload and leads to the development of failure in advanced age (Brooks et al. 1997).

On the other hand, Komatsu (Komatsu et al. 1995) has demonstrated that the 73-week-old SHR naturally developed all pathophysiological and clinical alterations that are associated with end stage renal disease (ESRD) in patients with essential hypertension. Decrease in total renal blood flow (RBF) along with structural and functional changes of the kidney of this rat strain further induces the progression of renal failure to ESRD (Mihailovic-Stanojevic et al. 2003).

Numerous studies confirmed the important role of the renin-angiotensin system in the domain of cardiovascular and renal physiology and pathophysiology. Angiotensin II (Ang II), the effector peptide of the renin-angiotensin system, exerts a variety of actions in physiological BP and body fluid regulation, and has also been implicated as a pathogenic factor, *via* angiotensin type-1 (AT1) receptor, in the development of cardiovascular (Pratt 1999; Unger 2001) and renal diseases (Hall et al. 1999). In ageing endothelial cells, production of vasoconstricting growth factors such as Ang II and endothelin-1 (ET-1) are increased, and that of vasodilatory factors (e.g. nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor) are reduced (Najjar et al. 2005). Previously we showed that losartan successfully protect kidney vessels against ageing and hypertension-induced dysfunction and remodeling by reducing hypertrophy and hyperplasia of vascular smooth muscle cells and by attenuating abnormal expressions α-SMA (an indicator of mesangial activation and renal fibrosis) (Mihailovic-Stanojevic et al. 2004).

Previous studies from others in young and adult animals have shown that hypertensive CV changes are reversible, while only a few studies with antihypertensive agents were processed in aged SHR (Susic et al. 1999a), being more or less effective in improving heart, renal, and vascular changes, depending on drags that were used and/or duration of treatment in those studies (Oddie et al.1993; Brilla et al.1996; Linz et al.1999; Cerbai et al.2000; Linz et al. 2000; Tsotetsi et al. 2001).

In this study we compared the effects of Ang II AT1 receptor antagonist, losartan on hypertension/ageing-related changes of systemic (systolic, diastolic, mean, pulse pressure and heart rate) and regional (carotid, aortal, and renal) haemodynamics, cardiac hypertrophy and biochemical parameters in adult and aged SHRs. We have now investigated whether 4-week losartan treatment could reduce hypertension and ageing-induced heart, renal and vascular changes, and thus decrease age-related CV risk in SHR.

Materials and Methods

Animals

Male SHRs, 9- and 18-months-old, obtained in this study were bred at the Institute for Medical Research (Belgrade, Serbia) and were descendants of breeders originally obtained through Taconic Farms (Germantown, NY, USA). They were maintained in temperature and humidity controlled rooms on a 12 : 12 h light/dark cycle. Animals were fed with standard laboratory chow (Veterinary Center Subotica) and water *ad libitum*. All experiments were done according to our Institutional guidelines for animal research and principals of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (Official Daily N. L. 358/1-358/6, December 18, 1986).

Experimental protocol

Animals were divided into four groups: untreated groups (9- and 18-month-old) received tap water, while treated SHR (9- and 18-month-old) received losartan dissolved in tap water (DUP 153, Du Pont, Wilmington, DE, USA; 10 mg/kg b.w./day) by gavage during four weeks of experiment.

Before haemodynamic measurements all animals were weight and placed in metabolic cages during 24 h for collection of urine for biochemical analysis and measurement of urine flow.

All procedures were done under general anesthesia (i.p. sodium-pentobarbital 35 mg/kg b.w.). Left femoral artery was cannulated by polyethylene catheter (P-50, Clay-Adams, Parsippany, NJ, USA) and connected to physiograph by low-volume displacement transducer (P23 Db; Statham, Oxnard, CA, USA) and a direct writing recorder (Physiograph Four; Nacro Bio System, Inc.) for continuous BP measurement. Systolic (SBP) and diastolic (DBP) blood pressure were measured directly, and mean blood pressure (MBP) was obtained by electronic integration, while heart rate was recorded at the same time. PP was displayed as the difference between SBP and DBP. For regional blood flow measurements left carotid artery was gently separated from the surrounding tissue. An ultrasonic flowprobe, 1RB (internal diameter = 1 mm) was placed around the artery and total carotid blood flow (CBF) was recorded using a Transonic T106 small animal flowmeter (Transonic System Inc., Ithaca, NY, USA). After abdominal incision renal artery preparation was utilized and RBF was recorded. Vascular resistance in these two vascular beds (carotid vascular resistance (CVR) and renal vascular resistance (RVR)) were calculated by dividing mean arterial pressure with total blood flow through

respective blood vessel, normalized for the body weight and expressed as mmHg·min·kg/ml. The segment of the abdominal aorta under the bifurcation of the renal artery was carefully prepared and aortal blood flow (ABF) was measured at the same procedure as for CBF and RBF, but with ultrasonic flowprobe, 2RB (internal diameter = 2 mm) and the aortal vascular resistance (AVR) was calculated as described above.

At the end of the study, blood samples were collected for the biochemical analysis. Urinary and plasma total protein and creatinine concentrations, and total plasma cholesterol and triglyceride levels were determined using Cobas Mira, Rosh analyzer and Cobas Integra 400 Plus (Elitech Diagnostic). Standard formula was used to calculate protein excretion, urine protein/creatinine ratio and creatinine clearance as estimation of renal function.

After that, animals were sacrificed and the heart was removed immediately. The atria were dissected free from the ventricles and discarded. The free wall of the right ventricle (RV) was separated carefully from the left ventricle (LV). Wet weight (g) of the both ventricles was measured, and corrected for the body weight and expressed as ventricular weight indexes (mg/g).

Statistical analysis

Results are expressed as mean ± S.E.M. One way analysis of variance (ANOVA) and unpaired Student's t- test was applied as appropriate. The differences between examined groups were considered being significant if $p < 0.05$.

Results

Haemodynamic parameters

Chronic losartan treatment significantly reduced SBP, MBP, and PP in the aged SHR. On the other hand, in the adult SHR, given losartan during four weeks resulted in slight, but not significant decrease in SBP, DBP and MBP without effects on heart rate and PP (Table 1).

CBF and CVR (Figure 1) were unaltered in adult SHR after losartan treatment. However, CBF was significantly lower and thus CVR was markedly higher in aged compared to adult untreated SHR. In the aged SHR, losartan has induced increased CBF and decreased CVR. Although, RBF was significantly lower in both untreated and losartan-treated aged, compared to adult SHR, but only in aged untreated SHR, RVR was significantly higher than in adult untreated group (Figure 2). Aortal blood flow was significantly increased and AVR (Figure 3) was significantly decreased in adult losartan-treated group compared to age match untreated SHR. At the same time, in the aged SHR, treatment with losartan slightly affects ABF, but resulted in the significant decrease in AVR. However, ABF was significantly lower and AVR higher in the aged losartan group then in adult SHR that received losartan.
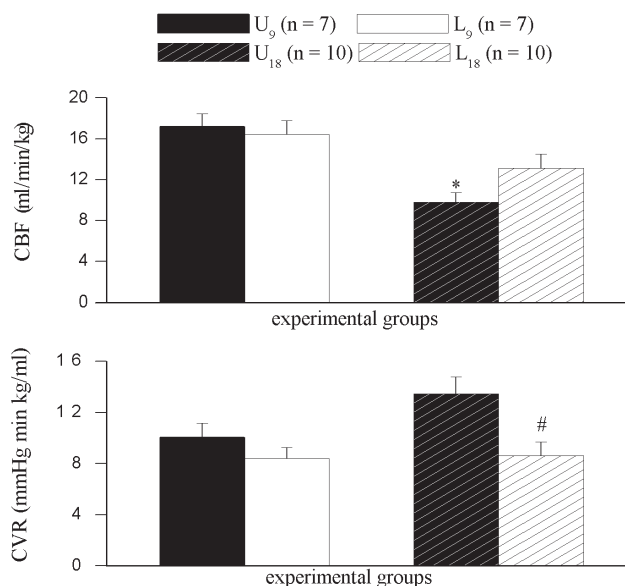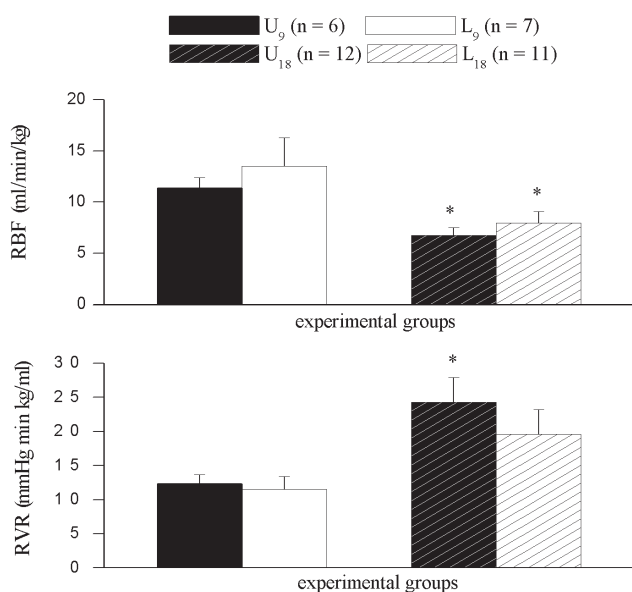

Figure 1. Carotid blood flow (CBF) and carotid vascular resistance (CVR) in SHRs. U_9 and U_{18}, untreated SHRs 9 and 18 months of age; L_9 and L_{18}, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; $^\#$ $p < 0.05$ vs. the age match untreated SHRs; * $p < 0.05$ vs. the adult SHRs.

Table 1. Systolic (SBP), diastolic (DBP), and mean (MBP) blood pressure, pulse pressure (PP), and heart rate (HR) in SHRs

	U_9 ($n = 6$)	L_9 ($n = 7$)	U_{18} ($n = 10$)	L_{18} ($n = 13$)
SBP (mmHg)	194.00 ± 10.91	174.43 ± 6.89	197.80 ± 8.84	147.85 ± 12.45 $^\#$
DBP (mmHg)	129.17 ± 13.15	110.86 ± 6.45	136.90 ± 8.94	98.46 ± 11.16
MBP (mmHg)	163.36 ± 17.07	133.32 ± 7.35	157.90 ± 9.14	114.15 ± 12.39 $^\#$
PP (mmHg)	64.83 ± 3.89	63.57 ± 5.53	60.90 ± 6.61	49.38 ± 3.76 *
HR (bpm)	388.33 ± 10.46	364.29 ± 7.82	347.40 ± 12.16 *	345.92 ± 8.81

U_9 and U_{18}, untreated SHRs 9 and 18 months of age; L_9 and L_{18}, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; $^\#$ $p < 0.05$ vs. the age match untreated SHRs; * $p < 0.05$ vs. the adult SHRs.

Figure 2. Renal blood flow (RBF) and renal vascular resistance (RVR) in SHRs. U_9 and U_{18}, untreated SHRs 9 and 18 months of age; L_9 and L_{18}, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; [*] $p < 0.05$ vs. the adult SHRs.
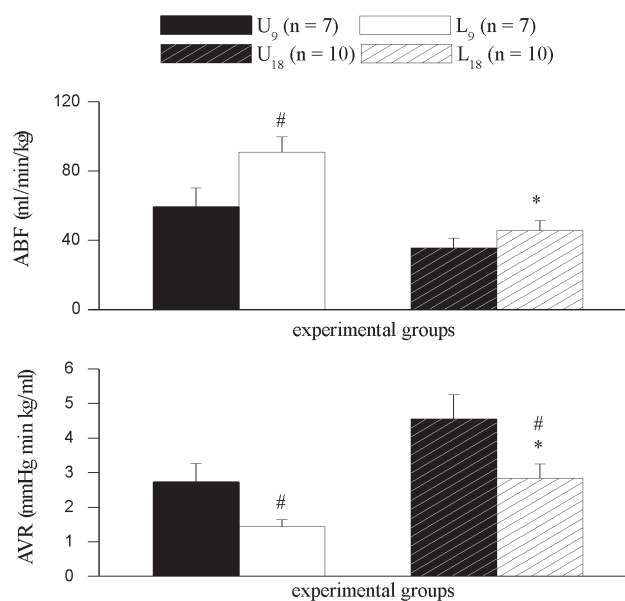
Figure 3. Aortal blood flow (ABF) and aortal vascular resistance (AVR) in SHR. U_9 and U_{18}, untreated SHRs 9 and 18 months of age; L_9 and L_{18}, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; [#] $p < 0.05$ vs. the age match untreated SHRs; [*] $p < 0.05$ vs. the adult SHRs.

Body and LV and RV weight

No difference in body weight was found among the experimental groups (Table 2). Left and right ventricular weight and ventricular weight indexes were significantly (LV: $p < 0.01$; LVI: $p < 0.001$; RV: $p < 0.05$; RVI: $p < 0.05$) lower in losartan-treated aged SHR than in untreated controls (Table 3). There were no differences in LV and RV weight and ventricular weight indexes between adult losartan-treated and untreated SHR (Table 3).

Biochemical parameters

Biochemical parameters are shown in (Table 2). The total plasma cholesterol and triglyceride levels were significantly increased in aged versus adult SHR, and losartan did not affect either of these parameters. Plasma creatinine level was significantly elevated, while urine creatinine concentration was significantly reduced in untreated aged, compared to adult SHR, thus creatinine clearance dropped significantly

Table 2. Body weight (b.w.), triglyceride (Trg), cholesterol (Chol), plasma creatinine (PCr) and urine creatinine (UCr) concentrations, total plasma protein (TP), urine flow (Uf), protein excretion (Pexc), creatinine clearance (CCr) and urine protein/creatinine ratio (UP/Cr) in SHRs

	U_9 ($n = 7$)	L_9 ($n = 7$)	U_{18} ($n = 10$)	L_{18} ($n = 13$)
b.w. (g)	302.86 ± 9.93	291.43 ± 13.17	308.50 ± 10.11	307.50 ± 9.26
Trg (mmol/l)	0.35 ± 0.04	0.38 ± 0.08	1.33 ± 0.2 [***]	1.32 ± 0.27 [*]
Chol (mmol/l)	0.70 ± 0.04	0.65 ± 0.05	1.28 ± 0.07 [***]	1.25 ± 0.09 [***]
PCr (μmol/l)	48.71 ± 1.34	51.57 ± 1.13	54.71 ± 1.99 [*]	54.40 ± 2.73
TP (g/l)	66.77 ± 0.99	73.47 ± 2.67 [#]	61.03 ± 4.06	55.66 ± 1.66 [***]
UCr (mmol/l)	10.56 ± 1.82	10.59 ± 1.64	3.22 ± 0.34 [***]	4.84 ± 0.55 [# **]
Uf (μl/min/kg)	23.36 ± 2.58	20.78 ± 2.47	47.07 ± 2.21 [***]	43.86 ± 2.56 [***]
Pexc (mg/min/kg)	0.18 ± 0.02	0.15 ± 0.02	0.39 ± 0.05 [**]	0.41 ± 0.08 [**]
CCr (ml/min/kg)	4.84 ± 0.85	4.01 ± 1.25	2.75 ± 0.27 [*]	3.84 ± 0.26 [#]
UP/Cr (mg/mg)	7.43 ± 0.77	6.55 ± 0.53	24.22 ± 2.85 [***]	17.69 ± 2.63 [**]

U_9 and U_{18}, untreated SHRs 9 and 18 months of age; L_9 and L_{18}, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; [#] $p < 0.05$ vs. the age match untreated SHRs; [*] $p < 0.05$, [**] $p < 0.01$, [***] $p < 0.001$ vs. the adult SHRs.

Table 3. Left and right ventricular (LV and RV) weight and weight indexes (LVI and RVI) in SHRs

	U_9 ($n = 6$)	L_9 ($n = 7$)	U_{18} ($n = 16$)	L_{18} ($n = 14$)
LV (g)	1.06 ± 0.10	1.09 ± 0.08	1.29 ± 0.04 [*]	1.14 ± 0.04 [##]
RV (g)	0.26 ± 0.06	0.24 ± 0.04	0.27 ± 2.02	0.22 ± 0.01 [#]
LVI (mg/g)	3.54 ± 0.34	3.74 ± 0.25	4.21 ± 0.09 [*]	3.65 ± 0.07 [###]
RVI (mg/g)	0.87 ± 0.18	0.82 ± 0.15	0.88 ± 0.06	0.71 ± 0.03 [#]

U_9 and U_{18}, untreated SHRs 9 and 18 months of age; L_9 and L_{18}, 9 and 18 months aged SHRs treated with losartan during four weeks of experiment; [#] $p < 0.05$, [##] $p < 0.01$, [###] $p < 0.001$ vs. the age match untreated SHRs; [*] $p < 0.05$, vs. the adult SHRs.

($p < 0.05$) in this group versus values in untreated adults. Chronic losartan treatment significantly increased urine creatinine concentration ($p < 0.05$), without changes in plasma creatinine, and therefore significantly increase glomerular filtration rate (GFR) measured by creatinine clearance ($p < 0.05$) in aged SHR. Urine flow, protein excretion and urine protein/creatinine ratio were also increased significantly with aging, and losartan decreased urine protein/creatinine ratio in aged SHR, but the difference was not significant.

Discussion

The data from the present study demonstrated that 4-week AT1 receptor blockade with losartan (10 mg/kg/day) improved BP, LVH carotid and aortic haemodynamics and preserved renal function and therefore decreased age-related CV risk in hypertensive rats. We also showed that Ang II is involved in RV remodeling in old SHR, because losartan treatment significantly reduced RV weight and RVI. Likewise, we demonstrated that in adult SHRs, when age-related cardiac and renal failure have not been completely developed yet, prolonged treatment with losartan resulted in marked but not significant reduction of SBP and MBP and improved haemodynamics in aorta.

Systolic hypertension and increased PP are independent risk factors for the development of cardiac hypertrophy (Zannad 1996; Izzo 2004). In the present study SBP, MBP and PP were significantly decreased after chronic losartan administration in aged SHRs. In addition to these beneficial effects on pressure overload, losartan reduced LVH that has been developed as a consequence of sustained hypertension and ageing. Susic and coauthors showed that angiotensin-converting enzyme inhibitor, enalapril, could reduce arterial pressure, total peripheral resistance, LV mass and ventricular fibrosis, without effect on RV mass in 65-week-old SHRs (Susic et al. 1999b). In the present study, however, we did not measure hydroxyproline content, as a marker of ventricular fibrosis, but our previous study confirmed the ability of losartan to improve cardiac performances by normalizing arterial BP, decreasing total peripheral vascular resistance and preventing LVH without significant reduction in myocardial and perivascular fibrosis in old SHRs (Mihailović-Stanojević et al. 2002). Thus, the reduction in pressure overload (afterload) could be, at last partly, sufficient to improve cardiac performances in aged SHR, suggesting that other mechanisms could account for myocardial fibrosis. Contrary to aged, in adult SHR LVH as an adaptive response to increased pressure overload, remain unchanged after losartan treatment, even marked but non-significant decrease in BP. In recent years, a large body of evidence demonstrated that Ang II acts not only through AT1 but also through Ang II type-2 (AT2) receptors that modulate the opposing effects of AT1 receptors on cardiac and vascular myocytic and fibrocytic mitogenesis as well as in cellular differentiation and arterial pressure regulation (Varagic et al. 2001b). This different response between adult and aged SHR could be explained due to enhanced AT2 receptor-mediated bradykinin and nitric oxide cascade during the AT1 receptor blockade (Siragy et al. 2000), Namely, there is an evidence that AT2 receptor mRNA is not expressed in detectable quantities in the adult heart, kidney, and vasculature, while its expression is increased in experimental heart failure, myocardial infarction, and vascular injury (Carey et al. 2000). Another explanation could involve the mechanisms that not include Ang II pathways, but is also responsible for pressure overload hypertrophy, such as endothelins or mechanical stretch (Harada et al. 1998).

The transition from hypertensive hypertrophy to heart failure in aged SHR is accompanied with marked changes of extracellular matrix composition, partly through transforming growth factor-ß1 regulating mechanisms and the mRNA for this growth factor was found to be enhanced in both ventricles of SHR with failing heart (Boluyt and Bing 2000). It is of interest that 4 week of AT1 receptor blockade reduces the right, as well LV weight to body weight ratio in advanced age, thus providing the evidence for losartan non-haemodynamic action in ventricular remodeling.

Decreased regional blood flow (CBF, RBF, and ABF) and increased vascular resistance of the respective vascular beds in the present experiment suggest the important role of ageing and hypertension in relation to the progression of vascular dysfunction. Vascular remodeling as a response to alterations in blood flow, pressure and atherosclerosis involves changes

in vascular smooth muscle cell growth, migration and vessel matrix alteration, and include mechanisms that increase production of vasoactive molecules such as Ang II and ET-1 (Berk 2001). Results obtained from our study agree that Ang II plays an important role in hypertension and age dependent vascular remodeling *via* type-1 receptors, thus the blockade of Ang II AT1 receptors with losartan significantly improved haemodynamics in aorta of adult as well aged SHR, however, AVR was still higher in aged than in adult SHRs, while haemodynamics in carotid artery was improved only in aged SHRs. Thus the beneficial effects of Ang II AT1 receptor antagonist on structure and function of the conductive arteries could account for the decline of CV risk in old rats.

Age-induced increase in total cholesterol and triglyceride levels, were not changed after 4 weeks of antihypertensive treatment with losartan. These results are in agreement with results obtained from study of Andrzejczak et al. (2007) who found non-significant difference in total cholesterol and high density lipoprotein level after enalapril, quinapril and losartan treatment in SHR.

We evaluated age-related kidney function through GFR and urinary protein/creatinine ratio as a marker of proteinuria (Krishna et al. 1987). Our results show that aging markedly reduced GFR in hypertensive rats. Chronic administration of Ang II receptor blocker, losartan, induced the significant increase in GFR in aged, without any effect in adult SHR. Tank et al. (1994) has obtained greater reduction in GFR and renal plasma flow in 15-month-old than in 3-month-old SHRs. They also found that responses to systemic vasoconstrictor stimuli, such as Ang II and ET-1, were exaggerated in the aging kidney, whereas responsiveness to losartan was preserved but not enhanced. Urinary protein/creatinine ratio was markedly elevated in both untreated and losartan-treated aged SHRs, as a consequence of renal disease progression, and losartan slightly attenuate age and hypertension-induced proteinuria. Taking all together, we conclude that protective effects of AT1 receptor blockade on renal function could be in part due to dilatory effects of Ang II blocker on efferent and afferent arterioles and augmentation of glomerular membrane permeability.

In summary, these results demonstrated that regional haemodynamics and LVH, as well GFR were significantly impaired in SHRs with advanced age. Moreover, presented data indicates that losartan within 4 weeks, beside its beneficial effects on haemodynamics, LVH and kidney function, also regresses RV weight, and therefore further decreases CV risk in aged hypertensive rats. This could contribute to better outcomes in elderly humans with hypertensive cardiac and renal complications.

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